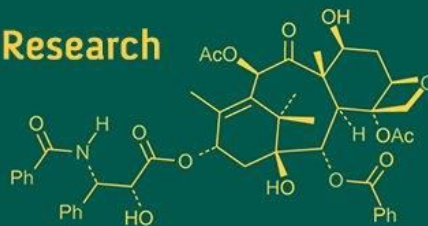
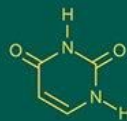
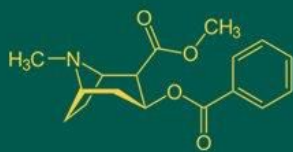


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Efficacy of monensin, multistrain probiotics, and probiotic-mineral-vitamin supplements on improving milk SNF and fat in crossbred cows under field conditions

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Abstract

The present study evaluated the effects of monensin, multistrain probiotics, and probiotic-vitamin-mineral supplementation, each administered with trisodium citrate, on milk yield and composition in dairy animals. Baseline values for milk yield, solids-not-fat (SNF), and fat percentage did not differ significantly among treatment groups. Following supplementation, the monensin-trisodium citrate group (T₁) showed a significant improvement in milk yield (2.9%) and SNF (6.6%), with minimal change in milk fat ($P \leq 0.05$). Animals receiving multistrain probiotics with trisodium citrate (T₂) exhibited significant increases in milk yield (3.3%), SNF (5.4%), and milk fat (15.4%), demonstrating the highest enhancement in fat percentage ($P=0.000$). Similarly, the combination of probiotics, vitamins, minerals, and trisodium citrate (T₃) resulted in significant improvements in all measured parameters, though to a lesser extent than T₂ for milk fat ($P=0.000$). Comparative analysis revealed that while all treatments improved milk yield and SNF, the greatest increase in fat percentage occurred in the probiotic-based groups. Overall, multistrain probiotics with trisodium citrate, followed by probiotic-vitamin-mineral therapy, proved most effective for enhancing milk yield, SNF, and fat, whereas monensin with trisodium citrate primarily improved yield and SNF.

Keywords: Milk yield, SNF, milk fat, Monensin, Probiotic, Multivitaminmineral, Trisodium citrate

Introduction

Dairy farming is a critical contributor to the livelihood and economic stability of Indian farmers and plays a significant role in the nation's overall economic growth. Rising population pressures have led to increased demand for milk and milk-derived products. To meet this demand, farmers primarily rely on exotic crossbred cattle; however, these animals often exhibit reduced milk fat and solids-not-fat (SNF) content, which poses challenges to farm profitability and the long-term sustainability of dairy production systems. To enhance both milk yield and the nutritional profile of milk, particularly its compositional quality, a variety of strategies have been adopted globally. These include dietary supplementation with probiotics, prebiotics, microminerals, vitamins, and selected feed-grade antibiotics as additives [1, 2, 3]. Probiotics, which are viable, non-pathogenic microorganisms, are often naturally present to some degree in the gastrointestinal tract. A wide range of bacterial and fungal species have been recognized for their probiotic potential, with commonly used genera including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Propionibacterium*, and *Saccharomyces*. Probiotics may enhance milk production and modify milk composition through several mechanisms: fiber hydrolysis, stabilization of rumen pH, improved feed conversion efficiency, enhanced nutrient absorption, increased production of volatile fatty acids (VFAs), and contribution of microbial protein to the host animal's nutrient supply [1]. Monensin is a carboxylic polyether ionophore that has been widely utilized in the dairy industry since receiving U.S. Food and Drug Administration (FDA) approval in 2004. Its mode of action involves the selective inhibition of gram-positive bacteria through disruption of ion transport, while concurrently promoting the metabolism of gram-negative microbial population [4]. This microbial shift increases ruminal propionate production and consequently reduces methane emissions. The enhanced propionate supply supports hepatic

gluconeogenesis, ultimately improving overall animal productivity. Trisodium citrate is an precursor of citrate, which is an intermediary product of TCA cycle, increase level of citrate indicator of positive energy and associated with low milk ketone bodies. Its helps to quick regeneration of mammary epithelium, which intern contribute for synthesis and secretion of milk solids [5].

Previous studies indicate that, under field conditions, numerous factors influence both milk yield and its compositional quality, including the availability of balanced feed, the provision of high-quality roughage, maternal characteristics, and environmental conditions. To address challenges associated with low milk SNF and fat content, various combinations of probiotics, prebiotics, and other feed additives have been investigated as potential interventions.

Materials and Methods

Milk samples from dairy animals presenting with low solids-not-fat (SNF) and milk fat content were evaluated at various milk co-operative societies across Uttara Kannada district, Karnataka, during 2023-2025. Routine testing was performed using an automated milk analyzer (Lactosure Eco 3.2). A total of 156 animals were identified for the study based on a 15-day average milk SNF value below 8.5% and or fat percentage below 3.5%.

The selected animals were randomly allocated into three treatment groups: T₁, T₂, and T₃.

T₁ (n = 58)

Animals in this group received Monensin sodium at 150 mg per day incorporated into feed, along with Trisodium citrate at 30 g per day per animal.

T₂ (n = 51)

Animals were supplemented with a multistrain probiotic formulation containing *Saccharomyces cerevisiae* (150,000 million CFU), *Saccharomyces boulardii* (25,000 million CFU), *Lactobacillus acidophilus* (22,500 million CFU), *Propionibacterium freudenreichii* (25,000 million CFU), and seaweed powder (50 g) per 500 g formulation. Each animal received 15 g of this probiotic daily, in addition to Trisodium citrate (30 g/day). The probiotic mixture was administered by mixing in concentrate feed after overnight soaking with 100-150 g feed and water.

T₃ (n = 47)

This group received 50 g per day of a multimineral-vitamin premix containing *Saccharomyces* and *Lactobacillus* species. The formulation included Vitamin A (700,000 IU), Vitamin D3 (70,000 IU), Vitamin E (250 mg), Nicotinamide (1000 mg), D.L-Methionine (1.929 g), L-Lysine (4.40 g), Copper (1200 mg), Cobalt (150 mg), Magnesium (6000 mg), Iron (1500 mg), Zinc (9600 mg), Iodine (325 mg), Selenium (10 mg), Manganese (1500 mg), Calcium (25.5%), Phosphorus (12.75%), Sulphur (0.72%), Sodium (5.9 mg), Potassium (100 mg), *Lactobacillus* spp. (1.5×10^{11} CFU), and *Saccharomyces cerevisiae* (30,000 million CFU) per kg of formulation, Trisodium citrate was also provided at 30 g per day per animal.

All supplements across the three groups were administered for a continuous period of one month. Milk production data, along with 15-day average SNF (%) and fat (%) values,

were recorded 3-4 days after the initiation of dietary supplementation to assess treatment efficacy.

Statistical Analysis

The collected data were statistically analyzed using SPSS software. Within-group comparisons of pre- and post-treatment values were performed using paired t-tests to determine the significance of treatment-induced changes in milk composition and yield. For evaluating differences among the three treatment groups (T₁, T₂, and T₃), a one-way ANOVA was employed, followed by appropriate post-hoc comparisons where necessary. Statistical significance was interpreted at the $P \leq 0.05$.

Results and Discussion

In the present study, the average 15-day milk yield, SNF%, and fat% before and after treatment were recorded and statistically analyzed (Tables 1-3). One-way ANOVA confirmed that animals selected for low SNF and fat percentages were randomly and uniformly distributed into the T₁, T₂, and T₃ groups prior to treatment, with no significant baseline differences.

Following treatment, the T₁ group supplemented with monensin and trisodium citrate showed a significant increase in milk yield (from 12.55 ± 0.60 to 12.92 ± 0.58 ; $P = 0.000$). These findings align with earlier studies reporting improvements in milk production attributed to enhanced rumen fermentation efficiency [6, 7]. The reported benefits are linked to increased glucogenic precursors such as propionate (C3), improved absorption of gluconeogenic amino acids, and reduced energy losses within the rumen environment [6]. Conversely, some studies in lactating cows and buffaloes have documented no significant effect of monensin on milk yield [2, 8], suggesting that the response may be dose-dependent and influenced by the level of energy precursor production.

Similarly, the SNF% increased significantly after treatment ($P = 0.000$), corroborating previous findings that associate SNF improvement with elevated milk solids including protein and lactose, likely driven by increased propionate production [2]. However, earlier work in buffaloes reported no significant change in SNF with monensin supplementation [8], indicating possible species- or physiological-status-related differences in response or dose dependent effect on milk solids [9].

A marginal increase in milk fat percentage was observed in the present study, consistent with some earlier reports [2]. Nonetheless, several studies have documented a negative effect of monensin on milk fat, suggesting that fat response may vary depending on dietary composition, stage of lactation, or monensin dosage. Percent increase in values depicted in bar diagram-1.

Animals under the T₂ group supplemented with multistrain probiotic and trisodium citrate exhibited significant increases in milk yield (3.3%), SNF (5.4%), and milk fat (15.4%) after treatment compared to before treatment ($P = 0.000$). These findings are in agreement with earlier reports demonstrating improvements in milk production and its constituents in purebred and crossbred cattle as well as goats [11, 12, 13, 14]. The observed enhancement in milk performance may be attributed to improved feed hydrolysis, greater nutrient availability and uptake, and increased production of volatile fatty acids (VFAs), which collectively elevate blood glucose levels. Probiotic supplementation also supports a

higher population of beneficial rumen microorganisms, leading to greater microbial protein synthesis. This microbial protein is ultimately converted into metabolizable protein, contributing to greater milk protein output and overall milk yield. Additionally, improvements in rumen pH and acetic-to-propionic acid ratios observed in this study further support the role of probiotics in enhancing milk fat content, as VFAs absorbed from the rumen serve as key precursors for milk synthesis [1, 15].

Animals in the T₃ group, which received a combination of probiotics, a multivitamin-mineral mixture, and trisodium citrate, exhibited a response pattern comparable to that of the T₂ group. Following supplementation, significant increases were observed in milk yield (3.5%), SNF (6.1%), and milk fat (8.2%) compared to pre-treatment values (P = 0.000). These results are consistent with previous field studies in crossbred cows, where probiotic-mineral supplementation resulted in a 12.06% increase in milk yield along with notable improvements in SNF and fat content [16]. Conversely, some studies have reported non-significant

changes in milk yield and SNF when selenium and probiotics were administered to indigenous and crossbred cows [17], suggesting that the response may vary depending on breed, baseline nutritional status, or specific mineral combinations used. Minerals and vitamins are integral to key physiological processes, including digestion, metabolism, and anabolic growth, and many are secreted into milk. Their supplementation can therefore enhance immune function and overall productivity, potentially contributing to the improved lactational performance observed in the T₃ group.

The trisodium citrate has been added in all group in order to improve milk quality in terms of somatic cell count and quick recovery of mammary cells as reported earlier [18]. Statistical analysis between treatment groups suggests that there was significant difference in increase in milk fat%, in which T₂ group recorded 15.4% followed by T₃ 8.21%, but no major fat shift in T₁ group (P=0.000). However, with respect to milk yield and SNF% no significant difference reported between treatment groups.

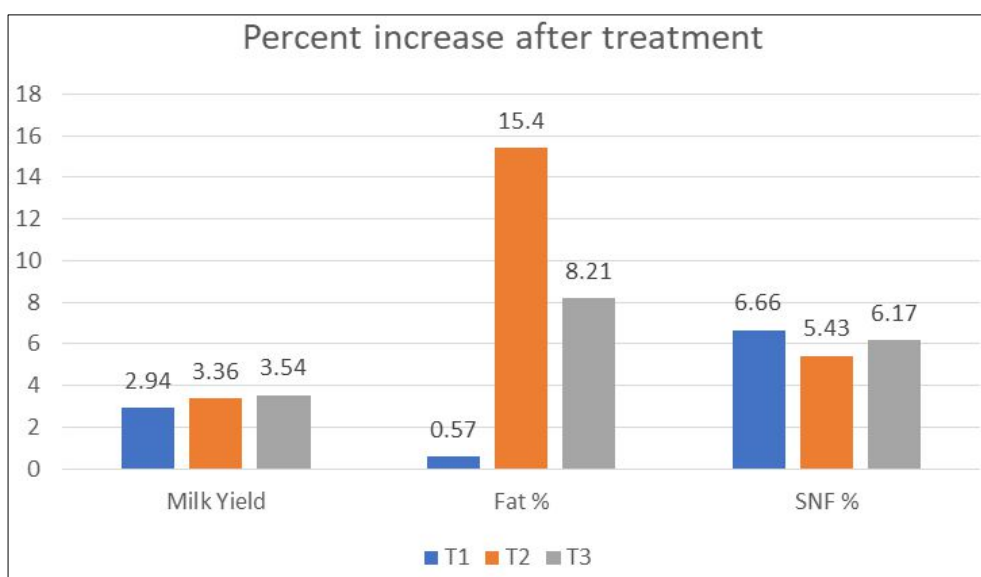


Fig 1: Percentage increase in milk yield, SNF and fat

Table 1: Paired t-test for comparison within group before and after treatment

Group	n	Milk Yield			Fat %			SNF %		
		Before	After	p value	Before	After	p value	Before	After	p value
T ₁	58	12.55±0.60 ^a	12.92±0.58 ^b	0.000	3.50±0.05 ^a	3.52±0.04 ^b	0.048	8.25±0.02 ^a	8.80±0.03 ^b	0.000
T ₂	51	11.88±0.29 ^a	12.28±0.27 ^b	0.000	3.57±0.06 ^a	4.12±0.06 ^b	0.000	8.28±0.03 ^a	8.73±0.03 ^b	0.000
T ₃	47	12.12±0.64 ^a	12.55±0.62 ^b	0.000	3.53±0.07 ^a	3.82±0.08 ^b	0.000	8.26±0.03 ^a	8.77±0.03 ^b	0.000

Means with dissimilar superscript (a, b) within a row of a parameter indicate significant difference

Table 2: ANOVA test between groups before treatment

Before the treatment	Milk Yield	Fat %	SNF %
T ₁	12.55±0.60	3.50±0.05	8.25±0.02
T ₂	11.88±0.29	3.57±0.06	8.28±0.03
T ₃	12.12±0.64	3.53±0.07	8.26±0.03
p Value	0.624	0.714	0.897

Table 3: ANOVA test between groups after treatment

After the treatment	Milk Yield	Fat %	SNF %
T ₁	12.92±0.58	3.52±0.04 ^a	8.80±0.03
T ₂	12.28±0.27	4.12±0.06 ^b	8.73±0.03
T ₃	12.55±0.62	3.83±0.08 ^c	8.77±0.03
p Value	0.634	0.000	0.070

Conclusion

The findings of the present study indicate that supplementation with monensin, multistrain probiotics, and probiotic-vitamin combinations enhances milk yield and solids-not-fat (SNF). Milk fat content increased specifically in the multistrain probiotic and probiotic-multimineral groups, while unchanged in the monensin group. Overall, multistrain probiotics in combination with trisodium citrate, followed by probiotic-multivitamin mineral with trisodium citrate therapy, appear to be the most effective strategies for improving milk yield, SNF, and fat content. Additionally, monensin combined with trisodium citrate may be used to further enhance milk yield and SNF.

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